REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested. Entry of this Amendment is proper under 37 C.F.R. § 1.116, because the Amendment places the application in condition for allowance for the reasons discussed herein; does not raise any new issue requiring further search and/or consideration; does not present any additional claims; and places the application in better form for an appeal should an appeal be necessary. Entry of the Amendment is thus respectfully requested.

I. STATUS OF THE CLAIMS

As correctly indicated in the Office Action Summary, Claims 1-60 are pending and are under consideration in the above referenced application. Claims 58-60 have been withdrawn from further consideration. Applicants cancel claims 5, 8 and 11-16 without prejudice or disclaimer as to the subject matter contained therein. No prohibited new matter has been introduced by this Amendment. Applicants reserve the right to pursue in a divisional or continuation application any subject matter canceled by way of this Amendment without prejudice or disclaimer.

Support for the amendments to the claims and the new claims is located throughout the specification and the claims as filed, particularly on page 3, lines 6-8, page 4, lines 20-27, page 6, lines 8-14 page 7, lines 9-14, and page 14, lines 20-25.

II. OBJECTION TO THE ABSTRACT

The Abstract was objected to for purportedly using phraseology often used in patent claims. Attached on a separate sheet please find a revised Abstract which does not make use of patent claim language. Thus, the objection to the Abstract is mooted.

III. THE SPECIFICATION

The Examiner requested that the specification be checked to correct minor grammatical and typographical errors. Applicants have amended the specification and corrected e minor errors. The amendments to pages 2, 3, 6-10, 13 and 15-18 do not introduce any prohibited new matter.

IV. REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 1-57 stand rejected under 35 U.S.C. §112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claim 1. Claim 1 stands rejected for reciting "by means of a first library of binding structure(s)". The phrase is purportedly unclear as to the means by which a binding structure is obtained. Claim 1 has been amended to add step (e), which clarifies the means by which a binding structure is acquired.

Claim 1 further stands rejected for reciting "other identifying information", because it is purportedly indefinite as to what is covered by this phrase. This phrase

is purportedly made more unclear by the use of the phrase "and/or". Claim 1 has been amended to remove the phrase "and/or". Applicants respectfully draw the Examiners attention to page 7, lines 9-14 of the specification, where there is a discussion of "other identifying information". Applicants also submit that one of skill in the art would know that in the context of the present invention that, "other identifying information" refers to information used to identify individual components of the libraries of the present invention. For example, a well known type of identifying information is chemical tags.

Claim 1 stands rejected for the recitation of "first" and "second" libraries, because the basis by which a library is considered a first or second library is purportedly not positively defined in the specification or the claims. Claim 1 has been amended to more clearly distinguish between a first and second library, as claimed in the present invention.

Claim 1 also stands rejected for the recitation of the term "authentic", because it is purportedly unclear what could be considered an authentic phenotype. The phrase "and representing the authentic phenotype" has been deleted from Claim 1.

Claim 1 stands rejected for the recitation of the term "characterized". The term purportedly renders unclear the process steps involved and is more applicable to a compound that is characterized by its properties. Claim 1 has been amended to replace the term "characterized" with "comprising".

For at least the above reasons and given the amendments to claims 1, Applicants respectfully asserts that the rejection of claim 1 is mooted. Claim 1, as amended, should be allowed.

Claim 4. Claim 4 stands rejected for the recitation of the terms "etc.", "single entity", "homogeneous", "uniform and/or other binding structures", because the metes and bounds of the claimed structures are purportedly unclear. Further, the terms "third" and "fourth library" purportedly lack antecedent basis. Claim 4 has been amended to delete the above terms and more clearly list different types of binding structures, and to correct the spelling of "homogenous". Additionally, claim 5 has been canceled to avoid redundancy with claim 4, as amended.

For at least the above reasons and given the amendments to claims 4, Applicants respectfully asserts that the rejection of claim 4 is mooted. Claim 4, as amended, should be allowed.

Claim 6. Claim 6 stands rejected, because it is purportedly not clear as to what is included by the phrase "previously uncharacterized, unpurified and unknown molecules". Applicants respectfully submit that the skilled artisan would know what is meant by "uncharacterized, unpurified and unknown molecules" in the context of the present invention. The binding structures of the present invention are obtained against target structures. The binding structures, as described in the specification and the claims, may be monoclonal antibodies, proteins or organochemical entities,

to name a few. The skilled artisan would know that the proper target structure would be any molecule appropriate for the specific binding structure.

In addition, Applicants respectfully direct the Examiner's attention to Tse et al. (WO 94/26787), which was cited in the outstanding Office Action. Tse et al. discloses a method for generating monoclonal antibodies directed against previously uncharacterized and unpurified antigens. See Tse et al. page 12. Applicants also turn the Examiner's attention to page 4, lines 25-27 of the instant specification, which includes a discussion of uncharacterized, unpurified and previously unknown molecules.

For at least the above reasons, Applicants respectfully asserts that the rejection of claim 6 is mooted.

Claim 7. Claim 7 stands rejected for the recitation of the term "expressed", because the term purportedly lacks antecedent basis. Further, it is purportedly unclear how the target structure is expressed when the gene includes only the binding structure. Claim 7 has been amended to replace "expressed" with "displayed" in order to more clearly convey that the claim does not teach expression of the target by DNA, as well as obviate the need for antecedent basis.

For at least the above reasons and given the amendment to claims 7, Applicants respectfully asserts that the rejection of claim 7 is mooted. Claim 7, as amended, should be allowed.

Claim 8. Claim 8 stands rejected for purportedly not further limiting base claim 1. Claim 8 has been canceled, and so this rejection is obviated.

Claims 11-16. Claims 11 and 14 stand rejected for purportedly lacking antecedent support, and for the recitation of the phrases "desired" and "undesired target structures". Claims 12, 13, 15 and 16 stand rejected for purportedly not further limiting base claim 1. Claims 11-16 have been deleted, thereby obviating the above rejections.

Claim 22. Claim 22 stands rejected as indefinite because the metes and bounds of a "portion or set of antigens" is purportedly unclear. Claim 22 has been amended to delete the above phrase and place the phrase "portion or a set thereof" and the end of the claim to clarify what is meant by "portion or set of antigens".

For at least the above reasons and given the amendment to claims 22, Applicants respectfully asserts that the rejection of claim 22 is mooted. Claim 22, as amended, should be allowed.

Claim 23 Claims 23 stands rejected as indefinite because it is purportedly unclear how the target can be based on a protein. Claim 23 has been amended to be dependent off of Claim 22. This amendment should more clearly illustrate that the target structure, although a protein-type structure, is often not the pure form of one structure, but a combination of several such structures.

For at least the above reasons and given the amendment to claims 23, Applicants respectfully asserts that the rejection of claim 23 is mooted. Claim 23, as amended, should be allowed.

Claims 24-27. Claims 24-27 stand rejected as indefinite because it is purportedly unclear how Claims 24-27 further limit the method of the base claim. Claim 24, the base claim for claims 25-27, has been amended to more clearly indicate how Claims 24-27 further limit the base claim. Specifically, claim 24 has been amended to more clearly disclose a specific embodiment of a method of obtaining tissue with the in vivo or in situ phenotype, i.e., histology. Claims 25-27 recite further limitations of one specific histological technique.

For at least the above reasons and given the amendment to claims 24, Applicants respectfully asserts that the rejection of claims 24-27 is mooted. Claims 24-27, as amended, should be allowed.

Claims 30 and 32. Claims 30 and 32 stand rejected as indefinite because it is purportedly unclear as to the difference between the "actively" and the "passively" secreted secretions. Applicants respectfully submit that the difference between active and passive secretions is well known to the skilled artisan. For example, passive secretions are secretions that are not normally, actively secreted, and can be produced by dying cells. For example, apoptotic cells passively release intracellular components, because these components are not normally or actively

secreted by healthy cells. In addition, Applicants respectfully turn the Examine's's attention to page 7, line 2 of the specification, which gives an example of an active secretion.

For at least the above reasons, Applicants respectfully asserts that the rejection of claims 30 and 32 is mooted. Claims 30 and 32 should be allowed.

Claim 39. Claim 39 stands rejected because it is purportedly unclear as to the standard of preselection of a library. Applicants respectfully submit that one skilled in the art would be able to determine the standard to preselect a library. Those of skill in the art know that a preselected library is one that is biologically biased by nature, and created through immunization or use of an organism. In addition, the specification at page 7, lines 3-8, which describes a preselected library as "preferably being a library produced by immunization against one or more target structures. However, the combinatorial and/or preselected library can also be a chemical library". With this information, one of skill in the art would be able to select the proper library for use with the present invention.

For at least the above reasons, Applicants respectfully asserts that the rejection of claim 39 is mooted. Claim 39 should be allowed.

V. REJECTIONS UNDER 35 U.S.C. § 102

For proving anticipation, "anticipation requires the presence in a single prior art disclosure of all elements of a claimed invention as arranged in the claims."

Jamesbury Corp. v. Litton Industrial Products, Inc. 225 U.S.P.Q. 253, 256 (Fed. Cir. 1985). Applicants respectfully traverse this rejection, because the cited references do not teach or suggest all of the elements of the rejected claims.

The claims of the present invention are directed to a method of acquiring one or more binding structures against a target structure through the use of a library of one or more binding structures linked to identifying information. This method can identify antibodies directed against antigens only expressed *in vivo* or *in situ*. Tissue sections can be used for the direct elution of phage particles. Further, the displayed target structure may be located in a cell membrane, intracellularly, extracellularly or intranuclearly.

1. Tse et al.

Claims 1-50 and 54 are rejected under 35 U.S.C. §102(b) as anticipated by Tse et al. (WO 94/26789). Tse et al. is cited for purportedly disclosing a method for generating an antibody directed against tumor antigens by incubating a combinatorial library of antibodies expressed on the surface of filamentous phage particles with target populations, including whole tissue sections.

Respectfully, Applicants traverse the rejection. The claims of the present invention teach the direct elution of phage particles. Tse et al. fails to teach or suggest the direct elution of phage particles. Specifically, Tse et al. disclose the use of tissue samples as an alternate method of gaining access to the cell surface of target cells. This procedure requires microdissection to prepare the target cells

before eluting the phage, rather than eluting the phage directly as in the present invention. The methods disclosed in Tse et al. also require copurification of the target cells together with the bound phage. The copurification is repeated through all steps and rounds of selection. Copurification is not disclosed or required by the present invention.

In addition, the claimed displayed target structure may be located in a cell membrane, intracellularly, extracellularly or intranuclearly. Tse et al. fails to teach or suggest that phage particles can be selected to structures located intracellularly or extracellularly. For at least the above reasons, Tse et al. does not anticipate the claimed invention. Thus, Applicants respectfully request withdrawal of the rejection of claims 1-50 and 54 under §102(b).

2. Cai et al.

Claims 1-50 and 54 stand rejected under 35 U.S.C. §102(b) as anticipated by Cai et al. (Proc. Natl. Acad. Sci. USA, Vol. 93, (June 1996) 6280-6285). Cai et al. is cited for purportedly disclosing the same method as Tse et al. Therefore, Cai et al. fails to anticipate the claims of the present invention for the same reasons that Tse et al. fails, as discussed above.

In addition, the claims of the present invention teach tissue section selection. Cai et al. discloses only intact cell cultures and fails to disclose tissue section selection. For at least these reasons, Cai et al. does not anticipate the claimed invention. Accordingly, Applicants respectfully request withdrawal of the rejection

Thus, Applicants respectfully request withdrawal of the rejection of claims 1-50 and 54 under §102(b).

3. Williams et al.

Claims 1-50 and 54 are rejected under 35 U.S.C. §102(b) as anticipated by Williams et al. (Abstracts from Immunotechnology, Vol. 2, No. 4, (1996) 295-296).

The Office Action cites the Williams et al. (Abstracts from Immunotechnology 2(4): 295-296, 1996) on page 10 of the Office Action under the section wherein the Examiner addresses rejections under §§ 102 and 103. No claims are indicated as rendered anticipated by or obvious in view of the reference. Morever, the reference is not applied to the claimed invention. Applicants are merely suggested to "[s]ee the entire abstract of the Williams reference, pp. 295-296."

Respectfully, Applicants that the Examiner must provide a more detailed explanation of the rejection. "It is important for an Examiner to properly communicate the basis for a rejection so that the issues can be identified early and the applicant can be given fair opportunity to reply." M.P.E.P. §706.02(j). "Where a claim is refused for any reason relating to the merits thereof it should be 'rejected' and the ground of rejection fully and clearly stated, and the word 'reject' must be used". M.P.E.P. §707.07(d).

However, in order to expedite prosecution, and without conceding to any possible basis for this rejection, Applicants respectfully submit that Williams et al. do not teach or suggest all of the elements of the present invention. Williams et al. is

an abstract of a meeting disclosing Fv regions formed from the libraries of autoimmune patients selected by *in vivo* blood circulation panning. In the present invention, the displayed target structure may be located in a cell membrane, intracellularly, extracellularly or intranuclearly. The blood circulation panning method of Williams et al. only yields binders to structures accessible from the vasculature and is therefore distinguishable. Thus Williams et al. can not anticipate the claimed invention. Applicants respectfully request withdrawal of the rejection of claims 1-50 and 54 under §102(b).

4. Ruoslathi et al.

Claims 1-50 and 54 are rejected under 35 U.S.C. §102(e) as anticipated by Ruoslathi et al. (U.S. Patent No. 5,622,699)). Ruoslathi et al. is cited for purportedly disclosing the same method of identifying antibodies from a phage display antibody as the present invention. Applicants respectfully submit that Ruoslathi et al. do not teach or suggest all of the elements of the present invention. In the present invention, the displayed target structure may be located in a cell membrane, intracellularly, extracellularly or intranuclearly. Ruoslathi et al. disclose a technique for the selection of phage particles, which is restricted to selection from the intravascularly accessible endothelial cell surface. Thus, Ruoslathi et al. can not anticipate the claimed invention. Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1-50 and 54 under §102(e).

5. O' Mahony

Claims 1-50 and 54 are rejected under 35 U.S.C. §102(e) as being anticipated by O'Mahony (U.S. Patent No. 6,117,632). The Office Action cites to page 10 of the O'Mahony reference ('632 patent). Like Williams *et al.*, no claims are indicated as anticipated by or obvious in view of the '632 Patent. Additionally, no mention is made as to what the '632 Patent may teach or suggest. Respectfully, Applicants submit that the Examiner must provide a more detailed explanation of the rejection, for reasons argued previously.

However, in order to expedite prosecution, and without conceding to any possible basis for this rejection, Applicants respectfully submit that O'Mahony do not teach or suggest all of the elements of the present invention. Specifically, the present invention does not involve transport across tissue and cell barriers. O'Mahony discloses methods of identifying peptide sequences which facilitate transport of drugs and other particles across barriers in viable cell, organ cultures and tissue cultures. Further, O'Mahony only discloses binders to structures that will transport binders after the application of voltage. The present invention does not require voltage for any step of the claimed methods. Thus, O'Mahony et al. can not anticipate the claimed invention. Applicants respectfully request withdrawal of the rejection of claims 1-50 and 54 under §102(e).

VI. REJECTIONS UNDER 35 U.S.C. § 103

Claims 1-50 and 54 were rejected under 35 U.S.C. §103(a), over Tse et al., Cai et al. or Williams et al. To make a *prima facie* case of obviousness, the Federal Circuit has articulated the proper analysis under 35 U.S.C. § 103 as follows:

> [W]here claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re* Dow Chemical Co., . . . 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure.

In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). It respectfully is submitted that a legally sufficient *prima facie* case of obviousness has not been adduced, because the cited references do not suggest that the methods claimed, let alone that the claimed methods could be conducted with a reasonable expectation of success.

1. Tse et al.

As explained above, Tse et al. is cited for purportedly disclosing a method for generating an antibody directed against tumor antigens by incubating a combinatorial library of antibodies expressed on the surface of filamentous particles with target populations, including whole tissue sections. Tse et al. do not teach or suggest that tissue sections can be used for direct elution, but rather requires a

cycle of copurification steps before eluting the phage. In addition, Tse et al. fails to suggest that the structures can be located extracellularly or intracellularly.

In fact, if the skilled artisan attempted to practice the claimed invention using the microdissection procedures disclosed in Tse et al., there would not be an expectation of success because the microdissection steps of Tse et al. would not be effective for selection. Accordingly, the methods of Tse et al. could not practice the methods of the present invention with a reasonable expectation of success.

As the claimed invention is not obvious over Tse et al., Applicants respectfully request withdrawal of the rejection.

2. Cai et al.

Cai et al. is cited for purportedly disclosing the same method as Tse et al. As such, Cai et al. fails to teach or suggest the claimed invention in the same way as Tse et al. In addition to the differences discussed above, Cai et al. discloses the selection of intact cultured cells and fails to disclose or even suggest the tissue sections of the present invention.

As the claimed invention is not obvious over Cai et al., Applicants respectfully request withdrawal of the rejection.

3. Williams et al.

The Office Action cites the Williams et al. (Abstracts from Immunotechnology 2(4): 295-296, 1996) on page 10 of the Office Action under the section wherein the Examiner addresses rejections under §§ 102 and 103. No claims are indicated as rendered anticipated by or obvious in view of the reference. Morever, the reference is not applied to the claimed invention.

It is the burden of the U.S. Patent and Trademark Office to establish a prima facie case of obviousness when rejecting claims under 35 U.S.C. §103. In re Reuter, 651 F.2d 751, 210 U.S.P.Q. 249 (C.C.P.A. 1981). However, in the interest of expediting prosecution, Applicants respectfully submit that Williams et al. do not teach or suggest the elements of the present invention. Williams et al. discloses Fv

regions formed from the libraries of autoimmune patients selected by *in vivo* blood circulation panning. In the present invention, the displayed target structure may be located in a cell membrane, intracellularly, extracellularly or intranuclearly. The blood circulation panning method of Williams et al. only yields binders to structures accessible from the vasculature. The skilled artisan could not expect to practice the claimed invention with success using a method limited to structures accessible only from vascular tissue.

As the claimed invention is not obvious over Williams et al., Applicants respectfully request withdrawal of the rejection.

VII. CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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ABSTRACT

The invention relates to a selection method and the products identified using the selection method which includes the steps of identifying one or more binding structures to a target structure using one or more libraries. The method includes the steps of reacting a first library with the displayed target structure to bind some of the binding structures to the displayed target structure, separating the displayed target structure and bound binding structures from unbound binding structures, and recovering bound or unbound binding structures. The bound or unbound binding structures can be amplified to create enriched libraries of binding structures. Binding structures thus identified are directed to target structures which are displayed *in vivo* and/or *in situ*.

Attachment to Amendment and Reply dated April 6, 2001

Marked-up Copy

Abstract

The invention relates to a selection method and the products [resulting from the methods, according to] identified using the selection method which includes the steps of identifying one or more binding structures [against] to a target structure [is obtained by means of a first library of one or more binding structures linked to genetic and/or other identifying information] using one or more libraries. The method [comprises] includes the steps of reacting a first library with the displayed target structure to bind some of the binding structures to the displayed target structure, separating the displayed target structure and bound binding structures from unbound binding structures, recovering bound or unbound binding structures[, and amplifying]. The bound or unbound binding structures, and can be amplified to create [a second] enriched [library] libraries of binding structures. [Identified binding] Binding structures thus identified are directed to target structures which are displayed *in vivo* and/or *in situ*.

Page 1, Title Beginning at Line 1

[IN SITU] *IN SITU* IDENTIFICATION OF TARGET STRUCTURES E.G. [IN VIVO] *IN VIVO* SELECTION METHOD FOR A PHAGE LIBRARY

Page 2, Paragraph Beginning at Line 18

The purpose of the present invention is to provide an extension of the application of phage technology for the selection of antibodies to complex antigens, thus making it generally applicable to identify antibodies directed against a number of important and/or novel target antigens and epitopes which are not accessible in *in vitro* culture systems, which would facilitate identification and dissection of antigens which are exclusively expressed [in vivo] *in vivo*. Of course, the method could generate antibodies to any target structure displayed within the tissue section.

Page 3, Paragraph Beginning at Line 24

FIG 1 is a histogram demonstrating yield (output/input of colony forming units, CFU) of C215 (black bars) and D1.3 (white bars) scFv phage and enrichment of specific phage at various amount of phage added in a volume of [100µl] 100 µl. The ratio, 1:15, of the C215 and D1.3 phage in the dilution series was kept constant.

Page 5, Paragraph Beginning at Line 5

In order to make the method more effective and specific the steps (a) through (c) as well as the steps (a) through (d) can be repeated. The amplification of bound binding structures can be obtained by means of synthesis in growing bacterial cells, PCR (polymerase chain reaction) synthesis, and chemical synthesis. Thus, monoclonal antibody, single-entity, homogenous, uniform and/or other binding

structures can be isolated and/or amplified from the second, or third, or fourth etc enriched library.

Page 6, Paragraph Beginning at Line 8

The displayed target structure can be obtained within a set of desired as well as undesired displayed target structures. In either case, bound structures or unbound structures are recovered in dependence of the selection intended. A selection system can thus be a combination of a tissue phenotype subtractive approach, i.e. the use of both positive and negative selection, with the use of different types of libraries, e.g. large naive or semi-synthetic libraries.

Page 6, Paragraph Beginning at Line 15

The target structure displayed *in vivo* and/or *in situ* and representing authentic *in vivo* and/or *in situ* phenotype is preferably obtained from tissue sections by a histological technique which comprises freezing and/or [fixation] fixing, and sectioning [of] a tissue sample. Such sections from a frozen tissue sample closely represent the original phenotype of all components at the moment the sample was frozen.

Page 6, Paragraph Beginning at Line 21

In [practise] practice, a tissue is frozen *in vivo* immediately after surgical removal from a human being or an animal, and the displayed target structure is

localized *in situ*. For the skilled man, there is no principal difference between a frozen tissue section and the same tissue *in vivo*.

Page 7, Paragraph Beginning at Line 1

from cells. The secretions can be secreted actively as well as passively. Actively secreted secretions are, for example, cytokines.

Page 7, Paragraph Beginning at Line 3

The first library of binding structure [is] according to the invention can be a [a] naive, synthetic, or semi-synthetic antibody library. It can also be a combinatorial and/or preselected library, the combinatorial and/or preselected library preferably being a library produced by immunization against one or more displayed target structures. However, the combinatorial and/or preselected library can also be a chemical library.

Page 7, Paragraph Beginning at Line 9

According to the invention, a first library or one or several binding structures is linked to genetic and/or other identifying information. Preferably, the linkage between such binding structures and genetic and/or other identifying information comprises particles of a filamentous phage or of any other virus. The linkage can also comprise polysomes or coded beads, i.e. beads identified by means of coding.

Page 7, Paragraph Beginning at Line 22

Pasqualini and Ruoslathi ([Nature] *Nature* 380: 364-366, 1996) [discloses] disclose a technique for selection of phage particles. Anyhow, this technique is clearly distinguished from the present invention in that it is profoundly restricted to selection towards the intravascularly accessible endothelial cell surface.

Page 8, Paragraph Beginning at Line 1

human peripheral blood samples were obtained from Lund University Hospital and Malmö General Hospital, Sweden. Human melanoma cell lines FM3 (kind gift from Dr. Jesper Zeuthen) and FMEX (ATCC) were used in FACS analyses. All tissues were snap-frozen in isopenthane, cooled in liquid nitrogen and stored at 70°C until cryostat sectioned. Six µm frozen section 3x4 mm wide were mounted on slides and air dried overnight (o/n).

Page 8, Paragraph Beginning at Line 8

The different phagemid vectors, all based on pBR322, were equipped with ampicilin or chloramphenicol resistance genes and a gene for the scFv-M13 pIII (residues 249-406) fusion protein expressed from the *lac* or the *phoA* promoter with the secretion directed by either the *ompA* of the ST II signal peptide. An amber stop codon allowed for production of soluble scFv molecules in non-suppressor strains. The fusion proteins contained either a recognition site for His64Ala subtilisin (Ala-Ala-His-Tyr) (17) or Restriction Protease Factor Xa (Ile-Glu-Gly-Arg) situated amino

terminal of pIII. An expression plasmid vector carrying a kanamycin resistance gene and the *lac* promoter was constructed for cassette insertion of scFv fragments in frame fusion with the superantigen staphylococcal enterotoxin A (SEA). The model scFV antibody constructs were derived from the C215 antibody directed to a well characterized epithelial antigen (the affinity for Fab binding is 2.3 nM) (24), and from the D1.3 anti-lysozyme antibody (25). For construction of a scFV antibody library, first strand cDNA was synthesized from total mRNA from lymph nodes of a Cynomolgus Macaque immunized with a suspension of pooled human malignant melanoma metastases mixed with alum adjuvant. Family specific sense primers annealing to the first framework region of human VH (IgG) and VL (lambda, only) genes and antisense primers annealing in the CH1 and CL regions respectively were used for the first PCR. ScFv genes, VL-$(Gly4Ser)_3$-VH, were assembled and inserted into [MIul] *MIuI* and [Xhol] *XhoI* sites of the phagemid vector. The phagemid pool was transformed to *E. coli* TG-1 cells. Three times 10^7 primary transformants were spread on minimal-agar plates, grown and pooled before superinfected with M13K07

Page 9, Paragraph Beginning at Line 4

Tissue sections were air-dried on slides, fixed in acetone at -20°C for 10 min and rehydrated in 20% fetal calf serum (FCS) in TBS in a humid atmosphere for 1 hour at room temperature. Model or library antibody phage in 100 ml 20% FCS were incubated at 4°C o/n. The slides were washed 6x10 minutes by gentle

agitation in 40 ml TBS in 50 ml Falcon tubes. Depending on the elution method, either of the following steps were performed: (i) Washes for 2x5 min in 50 mM Tris pH [7.6/1] 7.6, 1 M NaCl and 2 times in 1xPBS pH 7.6. Phage were diluted with 300 µl 0.1 M triethylamine for 15 min and neutralized with [150µl] 150 µl 1M Tris pH 7.4. (ii) Washes for 2x5 min in 1 M Nacl, 10 mM Tris-HCl, 6 mM $CaCl_2$, 1 mM EDTA, pH 8.0 (Ala-64 subtilisin buffer) or 100 mM NaCl, 50 mM Tris-HCL, 1mM $CaCl_2$, pH 8.0 (restriction factor Xa buffer). Bound phage particles were eluted by volume 300 µl 30 mg/ml mutant Ala64-subtilisin for 30 min or volume 300 µl 100 mg/ml restriction factor Xa (New England Biolabs, Beverly, MA) for 2 h. All washing and elution steps were performed at room temperature. Phage titres of incubation solutions and eluates were determined by counting antibotic resistant colony forming units (CFU).

Page 10, Paragraph Beginning at Line 5

Tissue sections first treated as described above were blocked with avidin 15 min and then with biotin 15 min diluted 1/6. Primary antibodies, scFv or scFv-Sea, were incubated for 1 h, followed by secondary 1 mg/ml affinity purified rabbit antibodies to the ATPAKSE tag peptide or 5 mg/ml rabbit antibodies to SEA for 30 min, and biotinylated goat anti-rabbit Mab diluted [1/1000] 1:1000 (Sigma) for 30 min, StreptABComplex HRP (DAKO) diluted [1/110] 1:110 in 50 mM Tris for 30 min. Between all steps the sections were washed 3 times in TBS. Antibodies, avidin and biotin were diluted in 20% FCS in TBS. The staining reaction was developed for 8 min in 0.5 mg/ml DAB (3,3'-diaminobenzidine tetrahydrochloride, Sigma) dissolved

in Tris pH 7.6 with 0.01 percent H_2O_2. The slides were rinsed 10 min in tap water and gradually dehydrated in 70-95 % ethanol and Xylene before mounting in DPX medium (Sigma).

Page 10, Paragraph Beginning at Line 19

FM3 or FMEX melanoma cells [200 000] 200,000 CELLS in 100 ml 1% BSA per tube, were incubated on ice [1h] 1 h with the primary antibody scFv K373-SEA (no primary antibody for the negative control), 30 min with 1 mg/ml rabbit anti-SEA Ig and 30 min with fluorescein-conjugated donkey anti-rabbit Ig (Amersham Life Science) diluted [1/100] 1:100. Two washes in 1% BSA followed each step. The samples were analyzed by FACS (Becton Dikinson).

Page 13, Paragraph Beginning at Line 4

Most antigens in a tissue section could be expected to distribute over only a fraction of the total tissue surface area (subpopulations of cells/substructures of tissue). As varying antigen distribution in individual tissue section was difficult to reconstruct for model experiments, a mosaic of antigen negative and antigen positive sections was constructed. Slides covered with 30 tissue sections of Colo205 SCID tumor and human spleen in various proportions were produced. A total amount of 3.6×10^8 scFv C215 phage and 3.0×10^{10} control phage was added per slide. The yield of specific phage increased linearly with an increasing number of antigen positive tissue sections, whilst the yield of scFv D1.3 phage was not

effected (fluctuated two-fold or less) by the different proportions of tissues within the experiment (FIG 2). When only antigen-negative (spleen) tissue was applied, the yield of phage of the two populations was similar. Thus, no difference in intrinsic non-specific binding between the phage stocks was seen. Unspecific binding of D1.3 phage did not differ significantly (less than two-fold) between other non-antigen expressing tissue, chosen to represent lipid-rich, epithelial parenchymal and mesenchymal organs (human brain, C. Macaque liver, human spleen and human heart, respectively (data not shown). The effect on enrichment of specific phage by reducing the antigen-expressing surface fraction was also exemplified by a four-fold decrease in yield of specific C215 phage after binding to a primary colorectal cancer biopsy containing epithelial tumor cell areas together with smooth musculature layers and connective tissue of the normal colon components (not shown).

Page 13, Paragraph Beginning at Line 29

The first selection round, which is aimed at rescue and enrichment of rare specific phage in a large library, is critically dependent on both the specificity of the procedure, i.e. the enrichment factor and the capacity scale of the

Page 15, Paragraph Beginning at Line 1

and K382, were unique and that all but one clone in the third round had a pattern identical to K378. The predominant representation of the K378 clone in the third round paralleled the increased phage yield at this stage. In a model experiment, the

scFv K378 phage was enriched 2490, 575 and 2 times over D1.3 sections of two different melanoma samples and of human spleen (not shown). The scFv antibody genes of K373 and K378 were re-cloned and expressed as fusion proteins with staphylococcal enterotoxin A (SEA) which was used as detection "tag" in immunoassays.

Page 15, Paragraph Beginning at Line 12

The scFv K373-SEA fusion protein was demonstrated by FACS analyses to bind to cultured cells of the FM3 (FIG 4A) and FMEX (not shown) melanomas but not to human peripheral blood lymphocytes (FIG 4B). The intensity of the staining indicated that the epitope was strongly and homogeneously expressed on viable cells and thus demonstrated the cloning of a cell surface reactive antibody phage through the use of a tissue-based selection method. The scFv K373-SEA but not the scFv D1.3-SEA fusion protein strongly and homogeneously stained melanoma cells in section of metastatic melanoma tissue (not shown). By FACS analyses, the K378-SEA fusion protein was found to bind only weakly to FM3 and not to FMEX or PBL. However, in tissue sections of metastatic melanoma this antibody bound strongly to both the melanoma cells and stroma components (not shown).

Page 15, Paragraph Beginning at Line 26

A mixture of filamenteous phage particles displaying the C215 scFv ($3.6x10^9$ particles) specific for an antigen expressed in all human epithelia, including small

intestine and large intestine (colon) and the 1F scFv displaying phage ($1.1x10^8$), reactive with only colon epithelium, was applied to frozen sections of small intestine or control (uterus) tissue in order to perform a negative selection step. After overnight incubation at [+4 C] +4°C, unbound

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phages in the supernatant were transferred to sections of colon epithelium in order to perform a positive selection step. After overnight incubation at [+4 C] +4°C, sections were washed six times for 10 min with 50 mM TRIS, ph 7.6 with 0.15 M NaCl. By incubation with Ala64-subtilisin (33 µg/ml) at RT, bound particles were eluted and used to infect *E. coli* DH5alphaF' bacterial cells. Infected bacterial cells were grown as single colonies on agar plates. [colonies] Colonies were counted and used to estimate the number of phage particles in the eluates. By using different antibiotic resistance genes for the two scFv phagemid constructs, the number of phage type could be estimated.

Page 16, Paragraph Beginning at Line 22

According to the method of the invention antibody phage can be directly selected in frozen tissue sections. The concentration of phage applied to the tissue sections influenced non-specific binding and efficiency of positive enrichment. Concentrations higher than [$5x10^9$/100µl] $5x10^9$/100 µl reduced yield of non-specific phage, indicating that a limited number of high-affinity non-specific binding sites

could be saturated at these concentrations. This was in contrast to low-affinity non-saturable sites responsible for a relatively constant yield (about 10^{-6} of input) of unspecifically bound phage particles within the high concentration range. Saturation of specific C215 epitopes could not be achieved even at the highest practicable phage con-

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centration, even though only fraction (<5 x 10^6) of the estimated number of available tissue epitopes (>10^{10}) were utilized for phage binding. Phages displaying antibody fragments (about 25 percent of the scFv C215 population by Western blot analysis, not shown) were present in large excess (>2000-fold) as compared to bound and eluted phage. At the highest phage concentration used, corresponding to ≅ 0.2 nM of C215 displaying phage, >6 percent of soluble antibody (affinity 2.3 nM) would be bound at equilibrium. This discrepancy suggests that other factors, e.g. steric hindrance and diffusion limitations, will be important to consider for further improvement of binding efficiency and selection capacity.

Page 17, Paragraph Beginning at Line 16

In single-pass experiments, enrichment of a few hundred times was routinely obtained, as compared to the relatively low enrichment factors (19 at the low end, Table 1) seen before optimization. After optimization, a useful enrichment of antibodies could be achieved even when using heterogenous samples with only 3

percent of antigen-specific surface area, mimicking cell subpopulations/substructures of a tissue (FIG 2). The million-fold enrichment achieved in three selection rounds on tissue sections was comparable to phage selection using cell suspensions (8, 12) and to use of cultured epithelial cells for enrichment of the same scFv C215 phage (not shown) and to pure antigen systems (1). For a given enrichment factor, the probability of rescuing rare specific phage will directly correlate to the number of eluted phage (which is limited by the total capacity of the selection system); this suggests that the high-end of the concentration range investigated (10^{10} - 10^{11} CFU per [100µl] 100 µl), should optimally be used.

Page 18, Paragraph Beginning at Line 16

Mabs to melanoma-associated antigens resulting from immunization of non-human primates have previously not been established, although primates have been used previously for tumor-immunization to produce polyclonal antibody reagents (19-21). Moreover, weak humoral and cellular immune responses to the autologous tumor in melanoma patients have clearly demonstrated the presence of melanoma antigens which are immunogenic to humans (22). By the choice of a primate rather than a rodent for immunization with human material, broad antibody responses to various normal human tissue components can be avoided (19). This allows the generation of a greater variety of more discriminating antibody

specificities, e.g. to tumor-associated antigens. However, any immunization procedure will be biased by the immune repertoire of the species used.

Attachment to Amendment and Reply dated April 6, 2001

Marked-up Claims 1, 4, 7, 22, 23, and 24

1. (Twice Amended) Method for acquiring binding structure(s) against a target structure by means of a first library of binding structure(s) linked to genetic [and/or] or other identifying information, [characterized by] comprising the steps of:

(a) reacting the first library with tissue sections comprising [a] an *in vivo* target structure [displayed *in vivo* and/or] or an *in situ* target structure [and representing the authentic phenotype to] such that the binding structures of the first library bind to the *in vivo* target structure or the *in situ* target structure [some of the binding structures to the displayed target structure];

(b) separating the *in vivo* target structure or the *in situ* target structure and [the displayed target structure and bound] binding structures which bound to the target structure from unbound binding structures; and

(c) recovering the bound or the unbound binding structures; and

(d) amplifying the bound or the unbound binding structures to create a second [enriched] library which is enriched with bound or unbound [of] binding structures; and

isolating the desired binding structure(s) against a target structure.

4. (Twice Amended) Method as claimed in claim 1, wherein the desired binding structure(s) comprise(s) monoclonal antibody(ies), proteins(s), peptide(s),

organochemical entity(ies), or any other homogeneous binding structure [characterized in that monoclonal, single-entity, homogeneous, uniform and/or other binding structures are isolated and/or amplified from the second, or third, or fourth etc enriched library].

7. (Twice Amended) Method as claimed in claim 1, characterized in that the displayed target is displayed [expressed] as an authentic phenotypic epitope.

22. (Twice Amended) Method as claimed in claim 7, characterized in that the displayed target structure comprises the whole and/or a portion and/or a set of (an) antigen(s), (an) epitope(s), (a) ligand(s), (a) receptor(s), (an) adhesion molecule(s), (a) matrix molecule(s) and/or (a) matrix associated molecule(s) and/or a portion and/or a set thereof.

23. (Amended) Method as claimed in claim 22 [1], wherein [characterized in that] the displayed target structure is based on protein, carbohydrate, nucleic acid, or lipid.

24. (Twice Amended) Method as claimed in claim 8, wherein the tissue section with the authentic in vivo or in situ phenotype is obtained by a histological technique [characterized in that the authentic *in vivo* and/or *in situ* phenotype authentic is obtained from tissue sections by a histological technique].